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The extraction of mimosine from ipil-ipil *(Leucaena leucocephala)* by soaking in water

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Leucaena leucocephala, otherwise known as ipil-ipil in the Philippines, leaves are used in cattle, poultry and swine feed and have been tried as a food ingredient in some fish diets (Glude, 1975). While ipil-ipil contains relatively high amounts of protein, its use as feed has been limited because of the presence of a toxic substance, mimosine, a lysine derivative, B N-(3 hydroxy-pyridone-4)-aminoproprionic acid (Yoshida, 1944).

To find a cheap and practical method of extracting mimosine, 50 g each of fresh leaves of local and Peruvian varieties were handpicked, separated from the stems and soaked in 250, 500, 750 or 1,000 mL of fresh tap water. The leaves were soaked for 24 hours with occasional stirring. After having determined the amount of water that would give maximum extraction of mimosine, leaves were soaked in 500 mL of tap water for 6, 12, 18, 24, 30, 36, 42 and 48 hours. After the soaking period, water was drained and leaves were airdried for one or two days depending on the prevailing weather condition.

Soaking leaves in water was a highly efficient method for the extraction of mimosine (Table 1). The amount of water used (250, 500, 750 and 1000 mL) did not significantly affect the amount of mimosine extracted from the local variety ($P < 0.01$). Similar results were obtained when Peruvian leaves were soaked in water up to 750 mL. Significantly less mimosine was extracted from the Peruvian leaves soaked in 1,000 mL of water ($P < 0.01$).

The longer the soaking time the more mimosine was extracted from the leaves. There was an exponential relationship between the amount of mimosine extracted and the duration of soaking for all the three varieties. Results show that more than 90% of mimosine was extracted from the leaves after soaking for 42 hours. When the leaves were soaked for more than 24 hours, there was a need to change the water to avoid fermentation of the leaves and a foul odor.

Mature leaves contain significantly less mimosine than immature leaves. Results showed that extraction of mimosine from immature leaves was significantly more difficult than extraction from mature leaves ($P < 0.01$) (Table 2). More mimosine was apparently extracted from mature leaves soaked in tap water than in distilled water, although no significant difference was noted. On the contrary, significantly more mimosine was extracted from immature leaves soaked in distilled water than in tap water ($P < 0.01$). When a mixture of leaves was soaked in either type of water no significant difference was observed in the amount of mimosine removed.

Table 1. **Mimosine content in *Leucaena leucocephala* leaves soaked in different amounts of water for 24 hours.**

Amount of tap water (ml)	Local ipil-ipil leaf meal Mimosine contents* (%)	Amount extracted (%)	Peruvian ipil-ipil leaf meal Mimosine content (%)	Amount extracted (%)
0	5.74^{a+}	– – –	3.34^{a}	– – –
250	1.26^{b}	78	0.51^{b}	85
500	1.33^{b}	77	0.51^{b}	85
750	1.29^{b}	77	0.54^{b}	84
1000	1.29^{b}	77	0.89^{c}	73

* Mean of two replicates

** Figures with the same superscripts are not significantly different from each other ($P<0.01$).

Table 2. Mimosine content in leaves soaked in 500 mL for various hours.

Length of soaking (hrs.)	Local ipil-ipil leaf meal Mimosine content (%)	Amount extracted (%)	Peruvian ipil-ipil leaf meal Mimosine content (%)	Amount extracted (%)	Hawaiian ipil-ipil leaf meal Mimosine content (%)	Amount extracted (%)
0	4.056 3/	--	3.837 3/	--	3.613 3/	--
6	4.385 1/	0	2.482 3/	35.13	3.475 2/	3.83
12	2.555 1/	44.41	2.420 2/	36.76	2.115 1/	41.47
18	1.362 2/	66.41	1.792 1/	53.16	1.230 1/	65.96
30	0.495 1/	87.79	0.385 1/	89.94	0.540 1/	85.06
36	0.250 2/	93.84	0.345 2/	90.98	0.432 2/	88.03
42	0.275 1/	93.22	0.385 1/	89.94	0.330 2/	90.87
48	0.145 2/	96.43	0.325 2/	91.51	0.390 2/	89.21

1/ Mean of two replicates

2/ Mean of four replicates

3/ Mean of three replicates

Results of biological evaluation of soaked and unsoaked local leaves showed that when unsoaked ipil-ipil leaves comprised 30% of the diet of juvenile *Penaeus monodon,* survival rate was zero after 8 weeks of feeding (Pascual and Tabbu, manuscript, 1979). On the other hand, 87% of the juveniles fed the diet containing local ipil-ipil leaves soaked for 24 hours survived. Thus soaking leaves for 24 hours was effective in reducing the mimosine content of the leaves and consequently improved survival rate. Soaking for 42 hours with a change in water after 24 hours of soaking would extract more mimosine than in 24 hours. Therefore, if by soaking for a longer period there is a reduction of mimosine, one may assume that the efficiency of the diet could be increased further.

Immature leaves in this study contained twice as much mimosine than mature leaves. However, protein content of young leaves was generally higher (27.50 to 37.75%) than the mature leaves (16.91%). Thus, although immature leaves contain higher protein, the presence of more mimosine in the young leaves and the difficulty in extracting up to 50% with tap water in 24 hours leads to the recommendation for the use of mature rather than immature leaves. Mixing one part mature and one part immature leaves by volume might help reduce the mimosine content and increase the protein content respectively. This will also provide for greater amounts of mimosine being extracted with tap water over purely immature leaves.

LITERATURE CITED

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